SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Morphology of Male Reproductive System of Two Solitary Bee Species (Hymenoptera: Apidae)

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ABSTRACT - Bees can form all levels of social organization, from solitary to advanced eusocial societies. Although 80% of the species exist as solitary species, most researches emphasize social species. This study focuses on the description of the male reproductive system of the solitary bees *Thygater analis* (Lepeletier) and *Melitoma segmentaria* (Fabricius) and searches for traits that support behavioral and phylogenetic studies. The reproductive system of males were dissected, fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.2 and post-fixed with 1% osmium tetroxide. The tissue was dissected and included for light microscopy. The species presented similar anatomical traits, including paired testicles, seminal vesicles, deferent ducts, accessory glands and an ejaculatory duct. Each testicle was composed of four follicles. In both species, a testicle and a seminal vesicle were surrounded by a joint capsule, forming a seminal vesicle-testicle complex. The two species presented accessory glands with very distinct morphological traits. The anatomic pattern and the histological traits of the reproductive system of the species studied were similar to those described for other bees. In addition, these traits can be used to differentiate species and allow inferences concerning their reproductive biology.

KEY WORDS: Accessory gland, histology, *Thygater analis*, *Melitoma segmentaria*

The bee fauna in the New World, especially in the Neotropical region, is among the richest in the world (Moure *et al* 2007). Bees have been intensely studied due to their economical and ecological importance; in many cases they are the exclusive pollinators of many flowering plants, including those cultivated by man. Apidae are also known as a diverse group with regard to their social behavior, with species representing all forms of social organization, from solitary to advanced eusocial (Michener 1974). In Brazil, among the estimated six thousand species, more than 80% are solitary (Silveira *et al* 2002).

In general, the insect's internal reproductive system is comprised of a pair of testicles, two deferent ducts, and one ejaculatory duct. In each deferent duct, there is a dilated region with modified epithelium – the seminal vesicle – where the sperm is kept until copulation. In addition, an accessory gland is connected to each duct. In Apidae, morphological variations of the reproductive system and the spermatozoa have been described (Snodgrass 1956, Cruz-Landim & Cruz-Hofling 1969a, b, Duvoisin *et al* 1999, Dallacqua & Cruz-Landim 2003, Ferreira *et al* 2004, Araújo *et al* 2005, Fiorillo *et al* 2009). Ferreira *et al* (2004), who examined 51 bee species (belonging to six families, according to the classification of Michener 1965), divided the male reproductive system into four types according to their anatomical differences. One remarkable example in bees is the absence of accessory glands associated with the reproductive tract, as seen in stingless bees of the Meliponini tribe.

Research regarding the male reproductive system histology of Apidae was carried out only in social bee species such as *Melipona quadrisfasciata* (Dallacqua & Cruz-Landim 2003), *Scaptotrigona xanthotricha* (Araújo *et al* 2005) and *Melipona mondury* (Lima *et al* 2006). This study represents the first histological description of the reproductive system of solitary bees, undertaken to understand the reproductive biology of this group as well as to supply traits that could be used in the systematics of Apoidea.

Material and Methods

Eight adult males from *Thygater* (*Thygater*) *analis* (Lepeletier) and eight from *Melitoma segmentaria* (Fabricius) were collected with entomological nets while they visited flowers of *Ipomoea* sp. (Convolvulaceae) at the campus of the Universidade Federal de Viçosa, in Viçosa, Minas Gerais State, Brazil.

Light microscopy. The reproductive systems of four males of each species were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 and post-fixed in 1% osmium tetroxide. They were then dehydrated in a series of increasing alcohol solutions and embedded in Historesin® (GMA, Leica). Semithin sections (2 µm) were stained with 1% toluidine borate and mounted in Entelan® (Merck). The analysis and photographs were made with an Olympus BX-60 microscope. For anatomic analysis, immediately after fixation, some of the reproductive systems were photographed using an Olympus BX-60 microscope and then sketched with pencil and Indian ink.

Results

The internal reproductive system in sexually mature males of solitary bees, *T. analis* and *M. segmentaria* consist of a pair of degenerated testicles with reduced volume, seminal vesicles, deferent ducts, accessory glands, and an ejaculatory duct. Each testicle contains four follicles. In both species, one testicle and the respective seminal vesicle are covered by a joint capsule, forming a seminal vesicle-testicle complex (Figs 1a,c, 2a, 3a). The general anatomy of the reproductive system is well-differentiated in these two species. In *M. segmentaria*, a capsule covers each seminal vesicle-testicle complex separately, and the seminal vesicles continue into the deferent ducts below the entrance of the accessory glands (Figs 1a,b). In *T. analis*, the capsule that covers each seminal vesicle-testicle complex is continuous, and extends to join the two complexes. Furthermore, in this species, the seminal vesicle joins the accessory gland, which is adjacent to the seminal vesicle-testicle complex (Figs 1c,d).

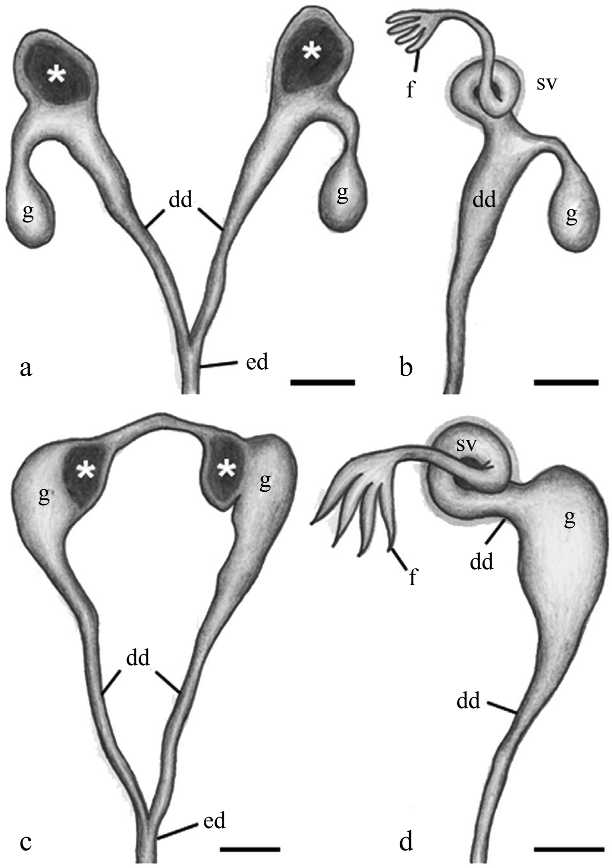

Fig 1 a) Male reproductive system of *Melitoma segmentaria*. The asterisk indicates the seminal testicle-vesicle complex and (b) shows one branch of the reproductive system without the capsule; c) Male reproductive system of *Thygater analis,* the asterisk indicates the seminal vesicle-testicle complex and (d) shows one of the reproductive system branches without the capsule. g = accessory gland, ed = ejaculatory duct, dd = deferent duct, sv = seminal vesicle, f = follicle. Bars: a-d = 100 µm.

The pre-vesicular deferent ducts have a simple epithelium, consisting of cubic cells with basal, spherical nuclei. In *T. analis*, some inclusions have been found at the basal region in addition to the nuclei (Fig 3b). The seminal vesicle is a specialized region consisting of a thick anterior region of the deferent ducts, in which the spermatozoa are stored until copulation. The seminal vesicle is very similar in both species (Figs 2a,c,d, 3a,c,d). It is tubular and presents a fold at the median portion that divides it into two regions, which lie parallel to each other and to the testicle (Figs 2c, 3a). In the seminal vesicle, the epithelium is simple, with prismatic cells with round, basal nuclei and several vesicles distributed throughout the cell (Figs 2c, 3c). A basal membrane separates the epithelial cells from a tunica of bundles of muscular cells (Figs 2d, 3c).

The seminal vesicle-testicle complex is connected to the post vesicular deferent ducts, which are filled with secreted fluids (Figs 2e, 3d). The post vesicular deferent ducts consist of simple epithelium of cubical cells with spherical, basal nuclei (Fig 2h).

Both species have accessory glands with clearly distinct morphological traits. In *M. segmentaria* they are round and the epithelium is made up of prismatic cells with spherical apical nuclei and contains many granules of secretion distributed throughout the cytoplasm (Figs 2f,g). In *T. analis,* the accessory glands are flattened ovals lined with low epithelium of cubical cells having fusiform, basal nuclei (Figs 3a,e).

Posteriorly, the two deferent ducts connect to the ejaculatory duct (Figs 2i,j) which has cubical epithelium, covered by a thin cuticle.

Discussion

The general morphology of the reproductive system of the solitary bees *T. analis* (Eucerini) and *M. segmentaria* (Emphorini) is similar to several other species of Hymenoptera. The reproductive system is particularly similar to pattern type III, observed in bees belonging to the Apinae subfamily (Ferreira *et al* 2004), characterized by the presence of a single capsule involving one testicle and its seminal vesicle, followed by well-developed accessory glands and a thick ejaculatory duct. Four follicles are counted in each testicle. Other subfamilies of bees, such as Mellitinae and some Megachilinae, also present four follicles per testicle (Roig-Alsina & Michener 1993, Ferreira *et al* 2004) with the exception of *Apis mellifera* L. which has around 250. The occurrence of three follicles was reported in more basal bees such as Colletinae, Andreninae and Hactinae (Ferreira *et al* 2004, Araújo *et al* 2005), and wasps Crabronidae (Moreira *et al* 2008), considered a sister group to bees. On the other hand, this number varies from one to 11 in Formicidae (Wheeler & Krutzch 1992) and in wasps Chalcidoidea, where only one

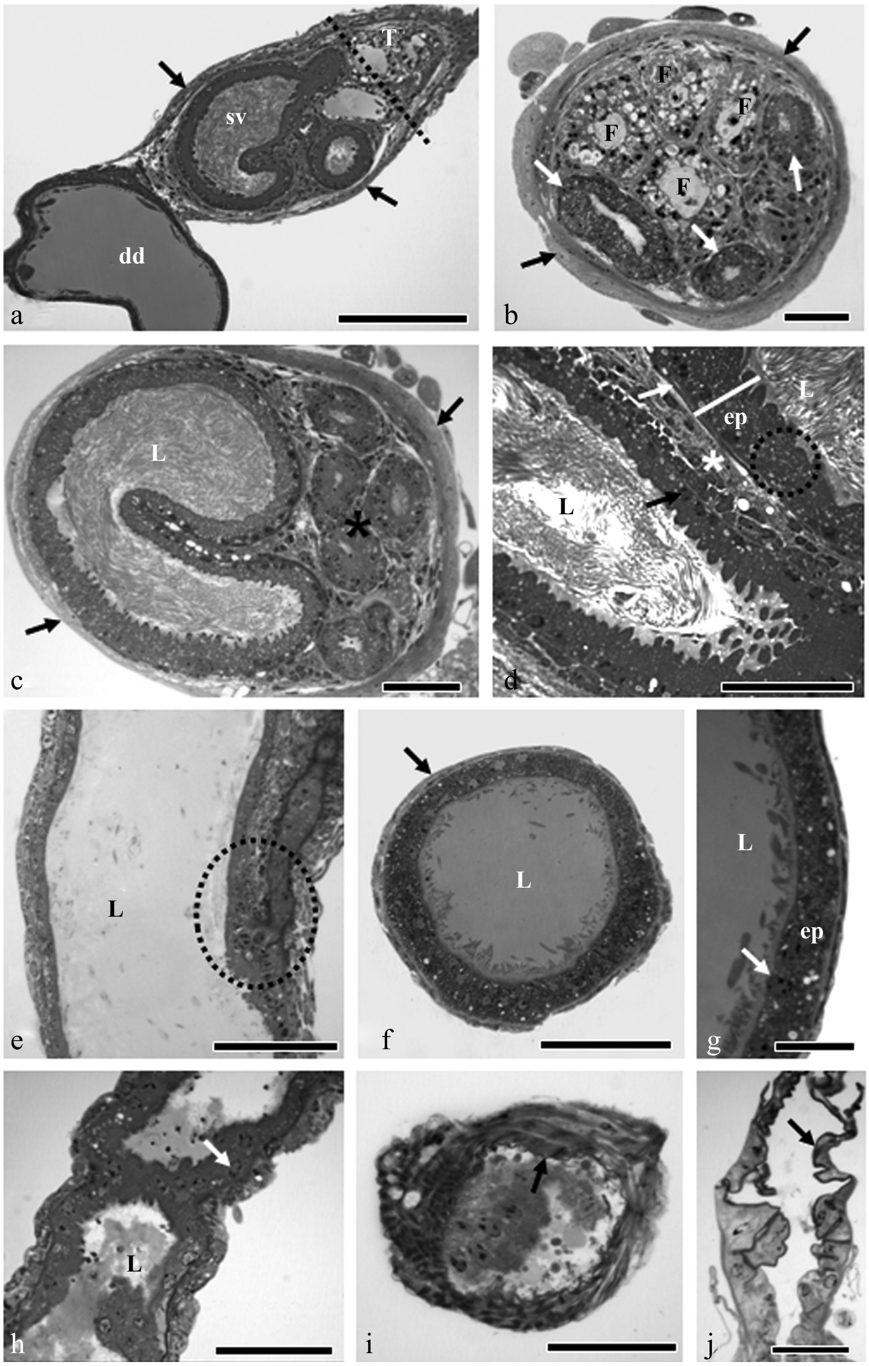

Fig 2 Histology of the reproductive system of sexually mature males of *Melitoma segmentaria*. a) Longitudinal sections of the seminal vesicle-testicle complex showing the degenerating testicle (T) and the seminal vesicle (sv) composed of a single capsule (arrows) and the deferent duct (dd); b) Transverse section at the level indicated by the dashed line of the previous figure, showing the four degenerating follicles (F), the pre-vesicular deferent duct (white arrows) and the capsule that covers the seminal vesicle-testicle complex (black arrows); c) Transverse section of the pre-vesicular deferent duct (asterisk) and the seminal vesicle with its lumen (L) containing sperm and the capsule (arrow); d) Transverse section of the seminal vesicle showing the epithelium (ep) comprised of prismatic cells with basal, spherical nuclei (black arrow) and cytoplasm with inclusions (dashed circle). The epithelium is separated by a muscular layer (asterisk) and the basal membrane (white arrow); e) Longitudinal section of the deferent duct where the seminal vesicle emerges (dashed circle), and its lumen (L) full of secreted fluid; f) Transverse section of the accessory gland, showing the lumen (L) full of secretions, and the muscular layer (arrow); g) Detail of the accessory gland tissue. Notice the epithelium (ep) formed by prismatic cells with spherical nuclei located at the apical third (white arrow). L = lumen; h) Longitudinal section of the deferent duct (dd) showing the epithelium of cubic cells with basal, spherical nuclei (arrow) and the secretion filled lumen (L); i-j) Transverse and longitudinal sections, respectively, of the ejaculatory duct showing the cuticle covered epithelium (arrow). Bars: a-c, f and i = 100 µm; d-e, h and j = 50 µm; g = 15 µm.

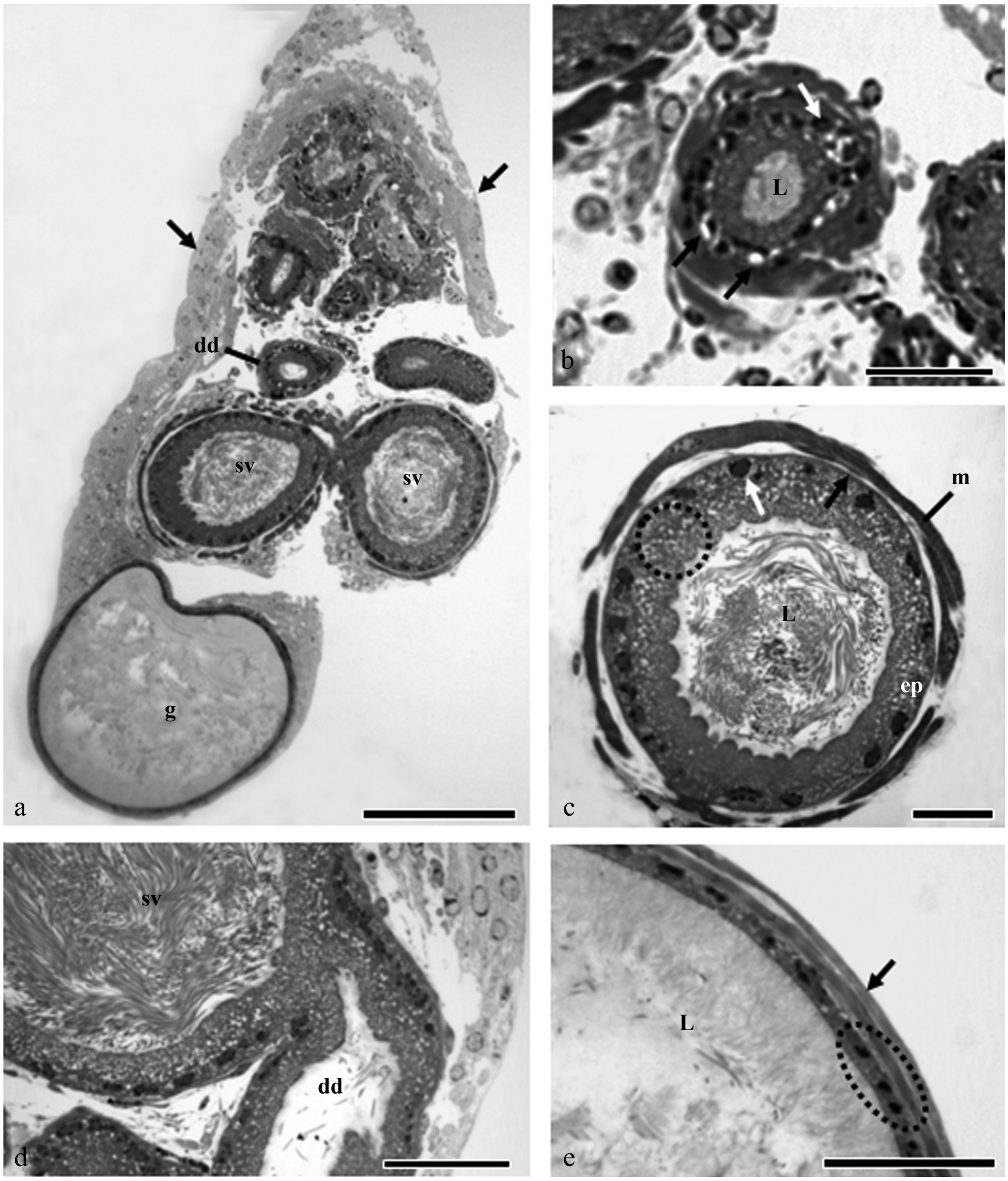

Fig 3 Histology of the reproductive system of sexually mature males of *Thygater analis*. a) Longitudinal section of the seminal vesicle-testicle complex showing testicles undergoing degeneration (T), the seminal vesicle (sv), and the pre-vesicular deferent duct (dd) surrounded by a single capsule (arrow) and the accessory gland (g) adjacent to the seminal vesicle-testicle complex; b) Transverse section in higher magnification of the pre-vesicular duct, evidencing the basal spherical nuclei (white arrow) and the presence of large inclusions close to the nuclei (black arrows). L = lumen; c) Transverse section of the seminal vesicle showing the lumen (l) filled with sperm and the epithelium (ep) comprised of prismatic cells with basal nuclei (white arrow). In their cytoplasm, inclusions can be observed (dashed circle). The epithelium is separated by a muscular layer (m) and a thick basal membrane (black arrow); d) Longitudinal section of the region where the seminal vesicle (sv) emerges in the pre-glandular duct (dd); e) Transverse section of the accessory gland, showing the lumen (L), full of secreted substance, the epithelium consisting in flattened cells with irregular nuclei (dashed circle) and the muscular layer (arrow). Bars: a = 100 µm; b-c and e = 25 µm; d = 50 µm.

follicle was found per testicle (Fiorillo *et al* 2008). The bees studied by Ferreira *et al* (2004) showed conspicuous variation in their reproductive system, even within the subfamily. In this study, the variation in the anatomic arrangement of structures along the reproductive tract allowed easy distinction between the two species studied, which could be considered a useful taxonomic trait.

In solitary bees, the sperm are produced at once and the testicles enter a degeneration process, which begins as soon as the bees become sexually mature – a fact also observed in social bees *Melipona bicolor* Lepeletier (Dallacqua & Cruz Landim 2003) and *Scaptotrigona xanthotricha* Moure (Araújo *et al* 2005), and in ants *Sonelopsis invicta* Buren (Ball & Vinson 1984). In these species, the spermatozoa leave the testicle in bundles and when they reach the seminal vesicles, these bundles become undone (Moreira *et al* 2004). Continuous sperm production is common in species that present long adult life and couple throughout this phase (Buschini 2007, Moreira *et al* 2008). Males of solitary bees, in general, emerge before the female and patrol the nidification areas and search for newly emerged females (Raw 2002). Thus, they need to complete their sexual maturation during pupation, before emerging.

The secretions of accessory glands are associated with the maintenance and activation of the sperm and with the induction and acceleration of oviposition in the female (Chen 1984, Raina *et al* 1994, Gillot 2003). Reduced female receptivity in insects is caused by several mechanisms, including substances secreted from accessory glands (Simmons 2001, Chapman & Davies 2004, Arnqvist & Rowe 2005). In *Drosophila melanogaster* Meingen, substances transferred in male ejaculates and accessory gland proteins, have been shown to reduce female sexual receptivity and longevity, and enhance egg production, ovulation and sperm storage (Wolfner 2002, Gillot 2003, Chapman & Davies 2004, Radharkrishnan & Taylor 2007). Several studies in other taxonomic groups report that mating often reduces female receptivity to subsequent mating, including hymenopterans (Boer *et al* 2009), hemipterans (Heady 1993, Himuro & Fujisaki 2008) and butterflies (Solensky & Oberhause 2009). In *T. analis* and *M. segmentaria,* these glands are well-developed in sexually mature males, where they are found full of secretions. Such secretions may be involved with the male reproductive strategy, contributing to the mechanism that guarantees copulation fidelity and keeps the sperm viable in the female's spermatheca for a longer period.

The ejaculatory duct is a single, median structure and presents a cuticle that shows its ectodermic origin, as in most insects (Bushrow *et al* 2006, Moreira *et al* 2008). The epithelium found in the spermatic ducts along the reproductive tract of bees is similar to that described for social bees (Snodgrass 1956, Cruz-Landim & Cruz-Hofling 1969a, b, Dallacqua & Cruz-Landim 2003, Araújo *et al* 2005, Lima *et al* 2006), ants (Ball & Vison 1984, Wheeler & Krutzsch 1992), and wasps (Dirks & Sternburg 1972, Bushrow *et al* 2006, Moreira *et al* 2008). However, some differences are evident, such as the large amount of inclusions in the epithelium of the seminal vesicle and the differences in the epithelium type in the accessory glands found in the two bee species studied.

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